would be expected from an additional wash of the cells. There was less loss when the cells were lifted with trypsin in Puck's saline containing no divalent ions (perhaps due to the shorter exposure necessary). Differences between colorimetric and isotopic data may reflect differences in the cells used and/or different sites damaged. Scraping can be expected to conserve extracellular material, *i.e.* cementing matrix, while causing damage to individual cells. Trypsinization may remove such extracellular material as well as alter the cells' permeability. This latter change must be reversible in many cases, however, as such cells are capable of dividing, whereas individual cells damaged by scraping can no longer do so. This loss of metabolic products could assume considerable importance in studies of cellular metabolism with isotopes.

Summary. HeLa cells and chick embryo fibroblasts were grown on glass and harvested under various conditions. Trypsinization and scraping were each compared with alcohol-ether denaturation. Trypsinization was found to be less efficient as a means of obtaining material for chemical analysis although less damaging to cell viability than scraping. Alcohol-ether denaturation *in situ* was the preferred means of harvesting cells for chemical analysis.

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Anti-heart Serum and Reactivity of Different Cell Types in Tissue Culture.* (24361)

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This report presents information regarding specificity of antisera and reactivity of cell types studied in tissue culture, to clarify the cellular mechanisms involved in the immediate hypersensitive reaction. In other work an antiserum to chick embryo heart cells was produced in guinea pigs and the cytologic reactions of chick heart fibroblasts in tissue culture to this antiserum were studied with phase (1) and electron microscopy(2). Antiserum was active at high dilutions, heat-labile, complement-like factors were necessary for its immediate cytotoxic actions, and it was inactive against mouse fibroblasts. Cortisol (17-hydroxycorticosterone) did not inhibit this cellular reaction. In the present report further evidence for species specificity and for lack of organ or cell type specificity of the antiserum is presented.

Methods. Fibroblasts and epithelial cells were grown in tissue culture by modification of methods previously used(1). The following nutrient solution was used after several were tried: human cord serum (Difco), 35%; chick embryo extract, 15%; and Hank's balanced salt solution, 50%. The cells were tested as before(1) with the exception that fresh normal guinea pig serum was added reg-

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ularly to maintain complement activity. Ring tests were used also in the attempt to demonstrate precipitins. Passive sensitization was attempted in several ways. 1) HeLa cells were exposed to chick embryo extract, washed, and then exposed to the antiserum. 2) HeLa cells were exposed to antiserum first and then to the embryo extract. 3) Chick embryo heart fibroblasts were exposed to bovine serum albumin, washed, and then covered with an antiserum which had been prepared by injecting guinea pigs with bovine serum albumin mixed with Freund adjuvants. The last procedure is known to produce high antibody levels(3), and the animals showed immediate skin reactions when tested with the protein alone. Attempts to grow capillary endothelium from chick bone marrow by the method of White and Parshly(4) were unsuccessful, as were attempts to grow smooth muscle from chick embryo intestine.

Results. The *antiserum* was quite stable, remaining active over 2 years when kept frozen and tested with fresh normal serum, but it lost activity in 2 months when diluted, even though kept frozen. Precipitating antibodies were not demonstrated. This confirms other evidence(5) that cytotoxic activity does not correlate necessarily with precipitating antibody.

Fibroblasts from chick embryo spleen and liver reacted with the antiserum as well as did chick heart fibroblasts. *Epithelial* cells from the intestine also reacted, demonstrating an antigenic similarity to heart fibroblasts. Like mouse fibroblasts, *malignant human epithelium* (HeLa cells) showed no reaction. This suggests that the antiserum is species specific.

Determination of conditions for *passive sensitization* outside the body of one or more cell types would aid greatly in understanding hypersensitive disease processes in the body. Failure to inhibit growth of cells from anaphylactically sensitized animals by exposing these cells to the sensitizing antigen in tissue culture had led Rich(6) to suggest that immediate hypersensitive reactions were mediated by endothelial and smooth muscle cells —2 types which could not be grown easily in tissue culture. However, considerations of the pathogenesis of rheumatic valvulitis, based upon evidence that mitral valve fibroblasts could take up foreign proteins(7), suggested that hypersensitivity might be passively transferred to other body cells if the proper conditions for adsorption of antigen or antibody on the cell surface could be found. This concept was strengthened by the production of an immediate hypersensitive reaction in isolated fibroblasts(1). Passive sensitization, in effect, has been demonstrated for isolated red blood cells(8). Passive sensitization of tissue complexes has been amply demonstrated in the body and in the Schultz-Dale bath.

When HeLa cells were exposed to chick antigen and then to antiserum, no reaction was noted. If the cells were exposed to the antiserum first and then to the antigen, only a transitory change in cytoplasm of some cells was noted. Perhaps low solubility of a complex antigen interfered with the results. After exposure of fibroblasts to purified soluble bovine serum albumin and then to specific antiserum, only slight cytoplasmic retraction was found.

This failure to obtain a well marked reaction stimulated efforts to determine the conditions for passive sensitization of endothelium or smooth muscle when isolated in tissue culture. Unfortunately several attempts to grow these cell types were unsuccessful.

Summary. 1) Further evidence for species specificity and for lack of organ or cell type specificity of an antiserum to chick embryo heart cells has been obtained from tissue culture studies. 2) Passive sensitization of cells in tissue culture could not be adequately demonstrated and attempts to test the reactivity of endothelium or smooth muscle were unsuccessful.

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Inhibition of Growth of Microorganisms by Benzimidazoles.* (24362)

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In microorganisms utilization of adenine or guanine is competitively antagonized by benzimidazole(1). The nucleotide moiety of Vit. B_{12} contains 5,6-dimethyl benzimidazole, and analogs of B_{12} may contain other benzimidazoles, purines, or triazoles(2). Certain substituted benzimidazoles inhibit growth of bacteria that require Vit. B_{12}, *e.g., Lactobacillus lactis*(3), *L. leichmannii,* and mutants of *Escherichia coli*(4). Indeed, inhibition of a mutant of *E. coli* by 4-nitrobenzimidazole or mercaptobenzimidazole can be reversed by Vit. B_{12}(4). The relation between benzimidazole inhibition and B_{12} metabolism should be studied with organisms whose specificity toward B_{12} is more rigid than that of these bacteria in which the B_{12} requirement can be by-passed with desoxyribosides or methionine. This paper is concerned with benzimidazole inhibition of growth of 2 algae which have the more specific B_{12} requirements and also of some gram negative bacteria that synthesize B_{12}.

Methods. For *Euglena gracilis,* strain Z, the media used for maintenance, vitamin depletion, and experimental studies were those used by Hutner *et al.*(5) for assay of B_{12}. *Ochromonas malhamensis* was maintained in the medium of Hutner *et al.*(6) and grown for experiments in a B_{12} assay medium(6). Algal cultures were incubated in the light at 25-26°C. *Rhizobium* species were maintained on yeast extract mannitol agar [medium 79 in (7)] in which Difco yeast extract, 0.5%, replaced yeast water; experimental cultures were grown aerobically at 30°C in mannitol, 1.0; Difco yeast extract, 0.5; K_2HPO_4, 0.05; $MgSO_4 \cdot 7H_2O$, 0.02; NaCl, 0.01; $CaCl_2 \cdot 2H_2O$, 0.0025; $FeCl_3 \cdot 6H_2O$, 0.001 (all g/100 ml medium). The 2-ethyl-5-methyl benzimidazole,‡ 5-methyl benzimidazole and benzimidazole were dissolved in absolute ethanol or acetone and added to media before autoclaving. Growth of cultures is expressed as optical density.

Results. Benzimidazole at 5×10^{-3}M concentration inhibited growth of *E. gracilis* (Fig 1) and *O. malhamensis* (Table I), both of which require B_{12}, and of 4 *Rhizobium* species (Table I), all of which synthesize B_{12} (8,9). This inhibition may, of course, be explained as due in part to interference with the utilization of purines though the results of Scott *et al.*(4) with *Escherichia coli* mutants imply that the purine site may be only of secondary importance.

The effects of 2-ethyl-5-methyl benzimidazole on growth of *E. gracilis* (Fig. 2) and *O. malhamensis* (Table I) appeared to be the same as those of benzimidazole. However, the rhizobia were more sensitive to 2-ethyl-5-methyl benzimidazole; they were inhibited by this drug at concentrations similar to those that inhibit synthesis of heme (Table I). The sensitivity of these bacteria to this drug is of interest because(11) the rhizobia are involved somehow in production of leghemoglobin in the root nodules of leguminous plants. *O. malhamensis* was more sensitive to a third compound, 5-methyl benzimidazole, than to either benzimidazole or the 2-ethyl-5-methyl derivative (Table I).

E. gracilis can utilize as a substitute for Vit.

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